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Hepatic glucose utilization and lipogenesis of hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) in response to dietary carbohydrate level and complexity

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Abstract

The influence of carbohydrate level and complexity on *in vitro* hepatic glucose utilization and lipogenesis were determined in hybrid striped bass, *Morone chrysops* ♀ × *Morone saxatilis* ♂. Six isocaloric, isonitrogenous diets containing glucose, maltose, or dextrin at two different levels (200 or 400 g kg^{-1} diet) were fed to adult fish for 15 weeks. Liver explants were obtained at near-maximum postprandial glycaemic response and incubated with radioactive labelled substrates. Glycogen synthesis from [^{14}C]glucose was not different among treatments and was less than $^{14}CO_2$ formation. $^{14}CO_2$ production increased as a function of carbohydrate level but was unrelated to carbohydrate complexity. There was no detectable conversion of [^{14}C]glucose to lactate for any treatment. Rates of *de novo* lipogenesis from [1-^{14}C]acetate were high in comparison to [U-^{14}C]glucose or [9,10-^{3}H]palmitate incorporation into liver lipids and differed in response to carbohydrate level and complexity. [9,10-^{3}H]palmitate esterification was an order of magnitude less than glycogen and CO_2 production but 4–10 times greater than [^{14}C]glucose incorporation into liver lipids. Palmitate incorporation did not differ among treatments. Incorporation of [^{14}C]glucose into liver lipids was higher in fish fed diets containing 400 g kg^{-1} carbohydrate. These data support the idea that glucose is not a major oxidative substrate in hybrid striped bass and indicate that the level of soluble carbohydrate should be limited to 200 g kg^{-1} diet or less for hybrid striped bass.

KEY WORDS: glucose utilization, hybrid striped bass, lipogenesis, MORONE

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Introduction

Striped bass (*Morone saxatilis*) and *Morone* hybrids are fourth in value and volume among domestically produced food fish (USDA/NASS 2006) and first in volume among U.S. recreational fisheries (NOAA/NMFS 2005). Farm gate prices have declined steadily, however, due to market pressure from increasing landings, domestic production, and imports of *Morone* bass (Carlberg *et al.* 2005). One strategy for maintaining a competitive edge is to reduce the cost of feed. Current diets for *Morone* spp. contain higher percentages of relatively expensive protein and lipid and lower percentages of relatively inexpensive soluble carbohydrate than feeds formulated for other livestock (Gatlin 1997). Fish appear limited in their ability to utilize dietary carbohydrate (Wilson 1994) and it is widely agreed that, with the exception of anoxia-resistant species (Krumschnabel *et al.* 2001), glucose has limited importance to fish metabolism (Hemre *et al.* 2002). Similar to most carnivorous fish, the most widely cultured *Morone* hybrid, sunshine bass (*Morone chrysops* × *M. saxatilis*), rapidly accumulates glycogen and fat in hepatic and intraperitoneal depots respectively (Rawles & Gatlin 1998). Sunshine bass also exhibit diabetic-like glycaemic response (Hutchins *et al.* 1998) when fed diets containing high levels of soluble carbohydrate. In contrast to striped bass (Small & Soares 1999), hyperglycaemia in hybrid striped bass is somewhat shortened and returns to basal levels within 15–16 h (Hutchins *et al.* 1998), suggesting a greater tolerance for elevated levels of carbohydrate in the diet. Attempts to promote lean growth and protein sparing in fish through manipulation of carbohydrate content of the diet have met with varying success (Wilson 1994; Hemre *et al.* 2002).

In order for dietary glucose to spare protein, glucose carbon must enter the Krebs cycle as pyruvate, be converted to acetyl-CoA, and be completely oxidized to CO_2 (Fig. 1). When energy intake is in excess of expenditure, or substrates

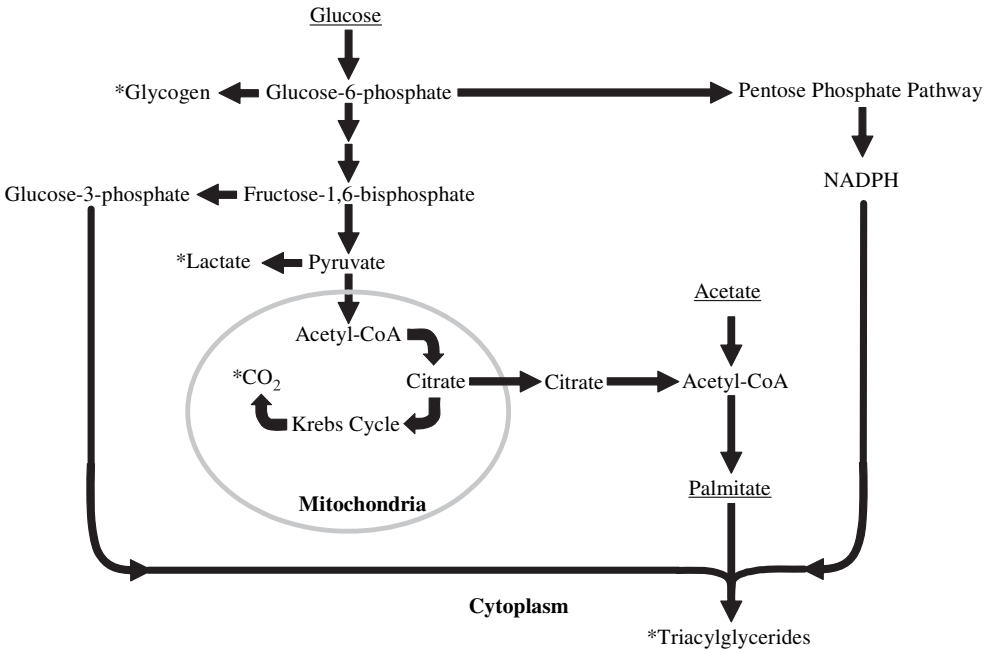

Figure 1 Pathways for glucose utilization and lipogenesis showing labelled substrates (underlined) and metabolic products (asterisk) measured in liver explants from adult hybrid striped bass fed diets containing glucose, maltose, or dextrin at two different levels (200 or 400 g kg^{-1}) of diet for 15 weeks.

other than glucose are preferentially catabolized, glucose utilization can follow several alternate paths that include: storage as glycogen; production of NADPH via the pentose phosphate pathway; esterification as glycerol backbone in triacylglycerides via conversion of glucose-3-phosphate; or export as citrate from the mitochondria, short-circuiting the Krebs Cycle, for synthesis of palmitate in the cytosol (Sul & Wang 1998). The liver is thought to be the preeminent site of lipogenesis in fish (Segner & Böhm 1994) and metabolic partitioning in this organ has not been noted in carnivorous fish. Hence, glycolytic and lipogenic flux can be measured by providing radiolabelled metabolic precursors, singly and in combination, to hepatic explants from fish that have been adapted to diets of varying carbohydrate level and complexity. The purpose of this study was to determine the influence of dietary carbohydrate level and complexity on hepatic glucose utilization and lipogenesis in hybrid striped bass.

Materials and methods

Animal, diets and feeding

A 3×2 factorial arrangement was employed in which six diets were formulated to contain one of three kinds of carbohydrate (glucose, maltose, or dextrin) at two different levels, 200 or 400 g kg^{-1}, of diet. Diets were maintained isocaloric at 13.4 MJ kg^{-1} of available energy (Nematipour *et al.* 1992) by adjusting lipid content to 110 and 20 g kg^{-1} of diet respectively (Table 1). Each diet was then assigned randomly to a tank containing eight individually tagged fish. Diets containing lipid at 20 g kg^{-1} of diet were analysed by gas chromatography to confirm that essential highly unsaturated fatty acids were at or above the known requirement level for hybrid striped bass (Nematipour & Gatlin 1993). Dietary protein was lowered from 400 g kg^{-1} (Brown *et al.* 1992) to 350 g kg^{-1} of diet to minimize energy utilization from protein. Menhaden fish oil (Zapata Haynie Corp., Reedville, VA, USA) was used as the lipid source. Each diet was mixed, pelleted, and stored as described previously (Rawles & Lochmann 2003).

Animal care and experimental protocols conformed to USDA/ARS Policies and Procedures 130.4 and 635.1 and policies of the TAMU Animal Care and Use Committee. Sunshine bass larvae obtained from a commercial grower (Keo Fish Farm, Keo, AR, USA) were reared to adults in earthen ponds at the Texas A&M University (TAMU) Aquacultural Research and Teaching Facility and then transferred to an indoor culture system where they were acclimated to experimental conditions. Fish were fed a maintenance diet for 16 weeks then pooled, individually tagged (Floy Tag & Manufacturing Co., Seattle, WA, USA), weighed (254 g average body weight) and distributed

Table 1 Composition (g kg^{-1} dry weight) of diets containing glucose (GLC), maltose (MAL), or dextrin (DEX) at two different levels (200 or 400 g kg^{-1}) of diet fed to hybrid striped bass

	Diet designation carbohydrate/lipid (g kg^{-1} diet)					
	GLC	MAL	DEX	GLC	MAL	DEX
Ingredient	200/110	200/110	200/110	400/20	400/20	400/20
Anchovy meal[1]	173	173	173	173	173	173
Casein[2]	235	235	235	235	235	235
Menhaden oil[3]	89	89	89	0	0	0
Vitamin premix[4]	30	30	30	30	30	30
Mineral premix[5]	40	40	40	40	40	40
Carboxymethyl cellulose[2]	40	40	40	40	40	40
Cellulose[2]	193	193	193	82	82	82
Glucose[6]	200	0	0	400	00	0
Maltose[6]	0	200	0	0	400	0
Dextrin[2]	0	0	200	0	0	400

[1] Omega Protein Corp., Hammond, LA, USA.
[2] US BioChemical Corp, Cleveland, OH, USA.
[3] Omega Protein Corp., Reedville, VA, USA.
[4] Vitamin premix (ICN Biochemicals, Inc., Aurora, OH, USA) contained the following (g kg^{-1} mix): ascorbyl-2-polyphosphate (Stay-C 35%), 10; thiamine mononitrate, 0.5; riboflavin, 3; DL-calcium pantothenate, 5; pyridoxine hydrochloride, 1; cyanocobalamine, 0.002; vitamin A palmitate (500 IU mg^{-1}), 0.2; DL-α-tocopheral acetate (1 IU mg^{-1}), 2; cholecalciferol (crystalline, 40 IU μg^{-1}), 0.002; menadione sodium bisulfite, 3.2; D-biotin, 0.05; choline bitartrate, 243; folic acid, 0.18; myo-inositol, 5; niacin, 5.
[5] Mineral premix (ICN Biochemicals, Inc., Aurora, OH, USA) contained the following (g kg^{-1} mix): aluminum chloride hexahydrate, 0.3; sodium chloride, 45; calcium carbonate, 3.4; calcium lactate pentahydrate, 348; calcium phosphate monobasic monohydrate, 133; cobalt chloride hexahydrate, 1; copper sulphate pentahydrate, 0.5; ferrous sulphate heptahydrate, 5; magnesium sulphate heptahydrate, 132; manganese sulphate monohydrate, 0.7; potassium iodide, 0.15; potassium phosphate dibasic, 239; sodium phosphate monobasic monohydrate, 88; sodium bicarbonate, 1; sodium selenate, 0.012; zinc sulphate heptahydrate, 3.
[6] Sigma-Aldrich, Inc. St Louis, MO, USA.

randomly into six tanks to form groups of eight fish per tank. Feeding rate was initially 3% of body weight day^{-1} (dry-matter basis) divided into two equal feedings (morning and evening) and was reduced gradually during the 15-week trial to 1% of body weight day^{-1} to maintain a level close to satiation without overfeeding. Fish in each tank were weighed individually every 2 weeks and rations adjusted accordingly. Weight gain, feed efficiency and survival were monitored throughout the trial.

The feeding trial was conducted in a closed recirculating culture system consisting of six 600-L rectangular tanks connected to a corrugated-plate settling chamber and biological filter. The settling chamber was emptied and rinsed on a weekly basis and the biological filter was emptied and rinsed once every 4 weeks. Water flow rate was maintained at approximately 5–7 L min^{-1}. Salinity was maintained at 5–7 g L^{-1} using a commercial seawater mixture (Fritz Chemical Co., Dallas, TX, USA) and well water. Water temperature was maintained at 24 ± 1 °C. Low pressure electrical blowers provided aeration via air stones and maintained dissolved oxygen (DO) levels at or near saturation. Water quality characteristics (DO, ammonia, nitrite and pH) were measured weekly to ensure optimum conditions for hybrid striped bass culture. An artificial diurnal cycle of 14 h light : 10 h dark was implemented by timed fluorescent lighting.

Composition of growth

Liver sections were dissected as described below and those that were not used for *in vitro* incubations were stored in plastic screw-cap tubes at −20 °C until proximate analyses could be conducted. Dry matter, crude protein and lipid in the liver were determined according to established methods (AOAC 1995). Liver glycogen was determined by the method of Hassid & Abraham (1957). Liver composition was expressed on a fresh-weight (wet-weight) basis. Calculated compositional indices included hepatosomatic index (HSI; liver weight × 100/body weight), intraperitoneal fat (IPF) ratio (IPF weight × 100/body weight) and muscle ratio (whole muscle weight × 100/body weight).

In vitro *incubations*

Ninety-minute *in vitro* incubations were performed on fresh liver sections for the determination of glucose utilization and *de novo* lipid and triacylglycerol biosynthesis according to Espinal *et al.* (1983) and Smith & Prior (1982) with

modifications as needed. At the conclusion of the feeding trial, fish were fed a final meal at suitable intervals and 6–8 h postprandial one fish was selected randomly from each treatment and killed by a quick blow to the head. Sampling intervals corresponded to near maximum postprandial glycaemic response (Hutchins *et al.* 1998). The intact liver was quickly excised, weighed, and placed in a glass Petri dish containing oxgenated (95% O_2 : 5% CO_2) Krebs–Henseliet bicarbonate buffer (KHB) and 5 mM glucose (pH 7.4) at 24 ± 1 °C (Wilson *et al.* 1992). The time required to select and incapacitate a fish and remove the liver did not exceed 3 min. Three sets of triplicate liver slices (9 total) of approximately equal size (100–200 mg) were dissected from each liver while immersed in oxygenated KHB. Subsequently, liver explants were sealed in individual incubation containers with different added substrates. Total time required to dissect explants and initiate all incubations from a single fish did not exceed 12 min. The above process was repeated six times to sample one fish per treatment per day (six fish per day; 36 total). Flasks for assays of glucose metabolism contained 0.5 μCi [U-^{14}C]glucose as the radioactive substrate. Vials for assays of lipogenesis contained either 0.5 μCi [1-^{14}C]acetate or 0.5 μCi [U-^{14}C]glucose and 0.5 μCi [9,10-^{3}H]palmitate as radioactive substrates. All isotopes were purchased from Amersham Life Science (Arlington Heights, IL, USA). Liver explants were incubated in a shaking water bath (90 min at 90 strokes min^{-1}) at the fish culture system temperature (24 ± 1 °C).

Glucose oxidation products

Isotopic CO_2 was collected and analysed as described by Smith (1983) with the following modifications. At the end of the collection period (90 min), hanging centre wells containing fluted filter paper for the collection of CO_2 were clipped into scintillation vials already containing 2 mL ddH_2O. Vials were sealed and vortexed until the filter papers released into solution. Scintillation fluid (15 mL, Biosafe II; Research Products International Corp., Mt Prospect, IL, USA) was then added to each vial and vials were resealed, vortexed, and allowed to stand overnight in the dark prior to counting to reduce photo- or chemiluminescence. Samples were counted on a LS 6000 Series (Beckman Instruments, Fullerton, CA, USA) scintillation counter using a dual isotope program. Analysis of isotopic glycogen was performed according to Espinal *et al.* (1983) with the following modifications. Liver slices in 1 mL 1*N* NaOH and 0.5 mL ddH_2O were repeatedly vortexed until disintegration. After the first addition of ethanol (75% vol vol^{-1}), glycogen was precipitated by centrifugation for 15 min at 1200 ***g*** and the supernatant fraction was discarded to remove cellular debris and unincorporated substrates. The resulting glycogen pellet was twice further purified by redissolving in ddH_2O (0.5 mL), precipitating in ethanol overnight, and discarding the supernatant fraction. The final pellet was prepared for counting by clipping the bottom conic section of the tube into a scintillation vial already containing 2 mL of ddH20. The sealed vial was vortexed until the glycogen pellet was completely dissolved prior to adding 10 mL scintillation fluid and remixing. Vials were allowed to stand overnight and counted as described above. Concentrations of isotopic lactate in the media were measured according to Smith & Freedland (1981). Rates of glucose utilization were expressed as nmol of substrate incorporated min^{-1} mg^{-1} liver (fresh-weight).

Lipogenic products

Neutral lipids were extracted from liver slices according to Folch *et al.* (1957) as modified by Smith (1983). Briefly, lipogenic reactions were terminated and liver slices were removed from the media, rinsed sequentially with buffer and salt solution under gentle suction to remove free lipid and unincorporated substrates. Slices were transferred to vials containing 15 mL of $CHCL_3$: CH_3OH (2 : 1, vol vol^{-1}), allowed to stand overnight, and vortexed until tissue disintegration to facilitate lipid extraction. An aqueous phase consisting of a 5% Na_2CO_3 solution was added to the vials, thoroughly mixed, and phase separation was achieved by centrifugation for 15 min at 2000 ***g***. Extractions were repeated three times to ensure removal of unincorporated substrates. Lipid samples were evaporated to dryness, resuspended in 10 mL scintillation cocktail and allowed to stand overnight prior to counting as described above. Rates of lipogenesis from [^{14}C]glucose or [^{14}C]acetate or esterification of [^{3}H]palmitate were expressed as nmol of substrate incorporated min^{-1} mg^{-1} liver (fresh-weight).

Statistical analyses

The software program GLM (SAS/STAT Version 7; SAS Institute, Cary, NC, USA) was used to conduct a factorial analysis of variance of the effects of three kinds of dietary carbohydrates (glucose, maltose and dextrin) at two inclusion levels (200 and 400 g kg^{-1} diet) on all response variables except feed efficiency. If treatment effects or interaction

between carbohydrate and level were significant ($P < 0.10$) for a particular response, differences among mean values were separated using Bonferroni *t*-tests (Miller 1981) for pair-wise comparisons to control type I error. Since feed was offered on a per tank basis, the effect of carbohydrate (two estimates per carbohydrate treatment mean) or level (three estimates per level treatment mean) on feed efficiency could be tested if tank effect was assumed negligible.

Results

Growth and composition of growth

Adult sunshine bass fed diets containing different carbohydrates at different levels more than doubled their weight over the course of the 15-week trial (Table 2). Initial average weight (254 g) was not different ($P = 0.998$) among dietary treatments (data not shown); however, after 15 weeks of feeding, interaction between dietary carbohydrate complexity and level was significant ($P = 0.003$) for weight gain. At 400 g kg^{-1} dietary carbohydrate, weight gains of fish fed the more complex carbohydrates (maltose or dextrin) were greater than those of fish fed glucose. Weight gains when carbohydrate was 200 g kg^{-1} diet were not influenced by carbohydrate complexity. Feed efficiency was not different among dietary treatments and ranged from 0.32 to 0.41 g gained g^{-1} dry diet fed.

Table 2 Performance of hybrid striped bass fed diets containing glucose (GLC), maltose (MAL), or dextrin (DEX) at two different levels (200 or 400 g kg^{-1}) of diet for 15 weeks[1]

Carbohydrate	Level[2]	Weight gain[3]	Feed efficiency[4]
GLC	200	152 ± 13 a	0.38
MAL	200	127 ± 16 a	0.38
DEX	200	138 ± 9 a	0.37
GLC	400	102 ± 11 B	0.32
MAL	400	166 ± 12 A	0.41
DEX	400	151 ± 14 A	0.37
Analysis of variance, *P*-values			
Carbohydrate		0.269	0.427[5]
Level		0.912	0.651[6]
Carbohydrate × level		0.003	——[7]

[1] Values are mean ± SEM of 7–8 fish per treatment. Mean values followed by different letters within carbohydrate level (lower case = 200; upper case = 400; g kg^{-1} diet) are different ($P < 0.10$).
[2] g carbohydrate kg^{-1} diet.
[3] Increase in initial weight (%); initial average weight was 254 g per fish.
[4] g weight gain g^{-1} dry diet fed. Feed efficiency was calculated on a per tank basis.
[5] $n = 2$ estimates of feed efficiency per carbohydrate.
[6] $n = 3$ estimates of feed efficiency per level.
[7] Insufficient degrees of freedom to calculate interaction.

Hepatosomatic index and IPF ratio values were greater in fish fed glucose at both 200 and 400 g kg^{-1} diet (Table 3). Values for IPF ratios were higher in fish fed the lesser (200 g kg^{-1}) amount of carbohydrate, i.e. the greater amount of lipid (110 g kg^{-1}). Conversely, muscle ratios were higher in fish fed the greater amount of carbohydrate (400 g kg^{-1}) and were lower in fish fed glucose at either level of diet. There were no differences in compositional indices of fish fed maltose or dextrin at either level in the diet.

Liver composition (fresh-weight basis) also was altered in response to dietary treatments (Table 4). Liver dry matter increased when carbohydrate level in the diet increased but was unaffected by carbohydrate complexity. Interaction between carbohydrate and level was significant ($P = 0.008$) for liver protein. At 200 g kg^{-1} dietary carbohydrate, livers from fish fed the simplest carbohydrate (glucose) exhibited the lowest concentrations of protein. At 400 g kg^{-1} dietary carbohydrate, livers from fish fed the most complex carbohydrate (dextrin) exhibited the lowest concentrations of protein. Liver lipid ranged from 117 to 169 g kg^{-1} (fresh-weight basis) and appeared unrelated to dietary treatment. Glycogen content of the liver ranged from 110 to 170 g kg^{-1} and was affected by both carbohydrate level and complexity. Livers from fish fed 400 g kg^{-1} dietary carbohydrate contained less glycogen than livers from fish fed 200 g kg^{-1} dietary carbohydrate. Moreover, fish fed maltose at 200 g kg^{-1} of diet exhibited the lowest concentration of glycogen in the liver, whereas fish fed maltose at 400 g kg^{-1} of diet exhibited the highest concentration of glycogen in the liver.

Glucose oxidation

Liver total glucose utilization and CO_2 production increased as a function of carbohydrate level in the diet but was unrelated to carbohydrate complexity in the diet (Table 5). The rate of ^{14}Cglucose incorporation into liver glycogen comprised 22–49% of total glucose utilization but was unaffected by either level or complexity of carbohydrate in the diet. There was no detectable conversion of [^{14}C]glucose to lactate for any treatment.

Lipogenesis

Rates of *de novo* lipogenesis from [1-^{14}C]acetate were quite high (181–578 nmol min^{-1} mg^{-1} wet tissue) in comparison to [U-^{14}C]glucose or [9,10-^{3}H]palmitate incorporation into liver lipids (Table 6). Rates of [1-^{14}C]acetate incorporation into

Table 3 Compositional indices (g kg^{-1} fresh-weight basis) of hybrid striped bass fed diets containing glucose (GLC), maltose (MAL), or dextrin (DEX) at two different levels (200 or 400 g kg^{-1}) of diet for 15 weeks[1]

Carbohydrate	Level[2]	HSI[3]	IPF ratio[4]	Muscle ratio[5]
GLC	200	26.7 ± 1.5 a	59.8 ± 1.8 a	456 ± 9 b
MAL	200	20.9 ± 0.9 b	49.9 ± 5.0 b	478 ± 7 a
DEX	200	21.6 ± 0.5 b	50.4 ± 3.4 b	473 ± 10 a
GLC	400	23.0 ± 1.0 A	46.7 ± 3.6 A	481 ± 5 B
MAL	400	21.2 ± 1.4 B	44.1 ± 3.6 B	500 ± 8 A
DEX	400	21.1 ± 0.5 B	44.4 ± 1.7 B	502 ± 8 A
Analysis of variance, P-values				
Carbohydrate		0.001	0.091	0.029
Level		0.135	0.005 (200 > 400)	<0.001 (200 < 400)
Carbohydrate × level		0.153	0.469	0.914

[1] Values are mean values ± SEM of 5–6 fish per treatment. Mean values followed by different letters within carbohydrate level (lower case = 200; upper case = 400; g kg^{-1} diet) are different ($P < 0.10$).
[2] g carbohydrate kg^{-1} diet.
[3] Hepatosomatic index (HSI) = liver weight × 100/body weight.
[4] Intraperitoneal fat (IPF) ratio = IPF weight × 100/body weight.
[5] Muscle ratio = whole muscle (fillet) weight × 100/body weight.

Table 4 Proximate composition of liver (g kg^{-1} fresh-weight basis) of hybrid striped bass fed diets containing glucose (GLC), maltose (MAL), or dextrin (DEX) at two different levels (200 or 400 g kg^{-1}) of diet for 15 weeks[1]

Carbohydrate	Level[2]	Dry matter	Protein	Lipid	Glycogen
GLC	200	379 ± 11	74.5 ± 2.6 b	141 ± 16	152 ± 8 ab
MAL	200	369 ± 12	93.4 ± 7.2 a	148 ± 13	131 ± 11 b
DEX	200	368 ± 6	82.1 ± 2.2 a	117 ± 14	170 ± 16 a
GLC	400	399 ± 13	89.3 ± 5.1 A	129 ± 18	111 ± 10 B
MAL	400	406 ± 13	82.4 ± 1.4 AB	169 ± 26	141 ± 14 A
DEX	400	395 ± 8	75.7 ± 2.0 B	160 ± 22	129 ± 6 AB
Analysis of variance, P-values					
Carbohydrate		0.554	0.193	0.387	0.252
Level		0.001 (200 < 400)	0.932	0.265	0.014 (200 > 400)
Carbohydrate × level		0.670	0.008	0.332	0.058

[1] Values are mean values ± SEM of 5–6 fish per group. Mean values followed by different letters within carbohydrate level (lower case = 200; upper case = 400; g kg^{-1} diet) are different ($P < 0.10$).
[2] g carbohydrate kg^{-1} diet.

liver lipids increased when carbohydrate level in the diet increased and were greater in livers from fish fed diets containing glucose at either inclusion level. Total lipid synthesis from [U-^{14}C]glucose was rather low and ranged from 6 to 15 nmol min^{-1} mg^{-1} wet tissue. Dietary carbohydrate level and not carbohydrate complexity increased rates of triacylglycerol synthesis from glucose. Rates of [9,10-^{3}H]palmitate esterification into liver total lipids did not differ with respect to dietary treatment; however, rates of esterification were four to ten times greater than rates of lipid synthesis from glucose.

Discussion

Final weights (600–800 g) and gains (100–170% of initial weight) during the 15-week study fell within the market size and expected growth for *Morone* bass stocked as advanced subadults (Harrell 1997). Only glucose at 400 g kg^{-1} of diet appeared to diminish weight gain whereas feed efficiency did not differ among treatments. In a similar study, Hutchins *et al.* (1998) found that both weight gain and feed efficiency in hybrid striped bass were reduced when fed 400 versus 200 g kg^{-1} dietary carbohydrate, regardless of carbohydrate complexity. Feed efficiency in that study also was higher for fish fed maltose or dextrin than in fish fed glucose. Hutchins *et al.* (1998) also reported that protein efficiency decreased at 400 g kg^{-1} dietary carbohydrate. Decreasing gain, as a per cent of initial weight, and decreasing feed efficiency are congruous with increasing size and reduced rates of intake in fish (Hepher 1988). Although growth in this study compares favourably to that observed in commercial settings with similarly sized fish, average initial weight (254 g) was much greater than that of Hutchins *et al.* (1998; 8.0 g). Feeding

Table 5 Liver [U-^{14}C]glucose utilization (nmol of substrate incorporated min^{-1} mg^{-1} liver; fresh-weight basis) in hybrid striped bass fed diets containing glucose (GLC), maltose (MAL), or dextrin (DEX) at two different levels (200 or 400 g kg^{-1}) of diet for 15 weeks[1]

		^{14}C-Labelled product			
Carbohydrate	Level[2]	CO_2	Glycogen	Lactate	Total
GLC	200	525 ± 108	236 ± 26	——[3]	761 ± 130
MAL	200	287 ± 71	278 ± 49	——	566 ± 64
DEX	200	216 ± 48	188 ± 22	——	404 ± 58
GLC	400	771 ± 246	259 ± 67	——	1030 ± 302
MAL	400	702 ± 146	199 ± 18	——	901 ± 157
DEX	400	530 ± 146	252 ± 67	——	782 ± 212
Analysis of variance, *P*-values					
Carbohydrate		0.137	0.789	——	0.197
Level		0.008 (200 < 400)	0.899	——	0.025 (200 < 400)
Carbohydrate × level		0.828	0.278	——	0.947

[1] Values are mean values ± SEM of 5–6 fish per group. Mean values followed by different letters within carbohydrate level (lower case = 200; upper case = 400; g kg^{-1} diet) are different ($P < 0.10$).
[2] g carbohydrate kg^{-1} diet.
[3] Not detected.

Table 6 Liver lipogenesis (nmol of substrate incorporated min^{-1} mg^{-1} liver; fresh-weight basis) in hybrid striped bass fed diets containing glucose (GLC), maltose (MAL), or dextrin (DEX) at two different levels (200 or 400 g kg^{-1}) of diet for 15 weeks[1]

		Substrate incorporation		
Carbohydrate	Level[2]	Acetate[3]	Glucose[4]	Palmitate[4]
GLC	200	406 ± 78 A	6.6 ± 1.6	53.6 ± 11.5
MAL	200	226 ± 42 B	8.2 ± 1.9	60.7 ± 18.2
DEX	200	181 ± 27 B	5.6 ± 0.6	53.2 ± 12.4
GLC	400	578 ± 145 A	10.9 ± 2.2	48.7 ± 6.1
MAL	400	372 ± 94 B	14.4 ± 6.4	58.3 ± 6.8
DEX	400	356 ± 63 B	15.2 ± 7.0	67.4 ± 13.0
Analysis of variance, *P*-values				
Carbohydrate		0.022	0.785	0.734
Level		0.020 (200 < 400)	0.042 (200 < 400)	0.811
Carbohydrate × level		0.982	0.780	0.689

[1] Values are mean values ± SEM of 5–6 fish per group. Mean values followed by different letters within carbohydrate level (lower case = 200; upper case = 400; g kg^{-1} diet) are different ($P < 0.10$).
[2] g carbohydrate kg^{-1} diet.
[3] Vials contained 100 mM glucose, 100 mM sodium acetate and 0.5 μCi [1-^{14}C]acetate as substrates.
[4] Vials contained 100 mM glucose, 7.5 mM sodium palmitate, 30 mg mL^{-1} BSA, 0.5 μCi [U-^{14}C]glucose, and 0.5 μCi [9,10-^{3}H]palmitate as substrates.

rates (1–1.5% of body weight day^{-1}) also were half to one third of those in the previous study (3–5% of body weight day^{-1}). Hence, the larger size and lower feeding rate may have attenuated the growth response typically seen in juvenile fish.

Composition of growth results indicated that intermediary metabolism in hybrid striped bass has a limited ability to be manipulated towards protein sparing and lean growth through changes in dietary carbohydrate. *Morone* spp. fed diets containing glucose grew less, accumulated less muscle, and stored more fat than those fed diets containing more complex carbohydrates, which confirms previous results (Hutchins *et al.* 1998; Rawles & Gatlin 1998; Small & Soares 1999). However, one confounding factor is that dietary lipid decreased from 110 to 20 g kg^{-1} of diet as dietary carbohydrate increased from 200 to 400 g kg^{-1} of diet, to maintain diets isocaloric and isonitrogenous. Therefore it appears hybrid striped bass more efficiently assimilated energy from lipid than carbohydrates since IPF ratio increased with increasing lipid content of the diet. Alterations in IPF deposition can, in part, explain alterations in muscle ratio as muscle ratio was inversely related to IPF level. The liver also stored excess energy in response to dietary manipulations. Glycogen plus lipid concentration of the liver exceeded 60% on a dry-weight basis. However, both glycogen and protein concentration differed with carbohydrate complexity and

level in the diet, whereas lipid content, which typically fluctuates at the expense of protein and glycogen, did not. The magnitude of change in HSI, however, was <0.5% on average between fish fed glucose and fish fed more complex carbohydrate and will equate to only small quantitative changes in energy storage relative to the whole fish. Similarly, IPF was responsive to complexity, but the magnitude of change was only 2% greater in fish fed GLC. In spite of differences in initial size, very similar trends in composition were observed in smaller hybrid striped bass fed identical diets (Hutchins *et al.* 1998). Intraperitoneal fat in cultured hybrid striped bass is typically 50–60 g kg^{-1} (Brown & Murphy 1991; D'Abramo *et al.* 2000), even in fish fed high-energy diets (Luzzana *et al.* 2002), and are rarely as high as 70 g kg^{-1} in *Morone* fed imbalanced diets (Nematipour *et al.* 1992; Webb & Gatlin 2003). Hence, *Morone* spp. can deposit significant amounts of lipid and some carbohydrate in visceral depots and a large portion of liver mass apparently can be partitioned for glycogen and lipid storage. Nevertheless, total storage capacity of liver is obviously limited. Maximum HSI was <30 g kg^{-1} of body weight in this study as well as in Hutchins *et al.* (1998) and is rarely as high as 50 g kg^{-1} in juvenile fish fed imbalanced diets (Keembiyehetty & Gatlin 1995). Obviously IPF constitutes a greater potential energy depot than liver and is responsive to dietary carbohydrate.

The high rates of [1-^{14}C]acetate incorporation into lipids observed in this study indicate that tissue explants are a viable *in vitro* method for measuring hepatic metabolism in *Morone* spp, and that lipogenesis is active in hybrid striped bass. Hybrid striped bass appear similar to most animals investigated in their ability to use acetate *in vitro* as a lipogenic precursor in the presence of glucose (Hansen & Ballard 1967; Segner *et al.* 1994). In contrast to glucose, lactate, or pyruvate, however, acetate is not a major lipogenic precursor *in vivo* for non-ruminants (Hansen & Ballard 1967). Segner & Böhm (1994) reported that liver is the preeminent site of lipid synthesis in fish. Incorporation of labelled glucose into glycogen, protein and lipids was highest in the liver among 13 tissues of the freshwater brown trout (Blascoe *et al.* 2001). Presumably, relative rates of metabolism in liver explants should be indicative of dietary glucose partitioning in hybrid striped bass.

Formation of CO_2 increased with increasing dietary carbohydrate level but lactate was undetectable after incubation of liver explants with [U-^{14}C]glucose. Identical results were achieved when multiple aliquots of media from this study were retested with fresh reagents (data not shown). Media and reagents used in this study were subsequently re-verified in independent incubations with explants of bovine liver where lactate was detected. It is possible that 90-min incubations may not be long enough to elicit a lactate response in hybrid striped bass liver explants. Segner *et al.* (1994) reported that lactate response was minimal in isolated rainbow trout hepatocytes during the first 5 h of incubation and the response only slightly increased after prolonged (20 h) culture. The absence of detectable lactate is more likely the result of rapid recycling of lactate to glucose to maintain steady-state conditions in the liver. Phillips *et al.* (1995) found that steady-state levels of lactate were achieved in isolated rat hepatocytes over a wide range of glucose concentrations, not by cessation of glycolysis but rather, through conversion of lactate to glucose at rates equal to lactate formation.

The contrast between *in vitro* glycogen production and total liver glycogen concentration in response to the dietary treatments is notable. Glycogen concentration was higher in the livers of fish fed the higher lipid level (110 g kg^{-1} of diet) and more complex (dextrin) carbohydrate; whereas, *in vitro* glycogen synthesis was not different among treatments and about one-half to one-third that of CO_2 formation. In the current study, lower dietary carbohydrate (200 g kg^{-1}) was associated with higher dietary lipid (110 g kg^{-1}) and vice-versa. These data suggest the higher lipid content of diets containing 200 g kg^{-1} carbohydrate inhibited glucose oxidation and increased glucose storage and that these fish probably were in a state of glycogen homeostasis, stores being replete, at the time of sampling. High dietary lipid would be expected to inhibit *de novo* lipogenesis from glucose, partition glucose towards glycogen storage, enhance fatty acid oxidation and diminish triglyceride synthesis (Hue *et al.* 1988; Jump & Clarke 1999), particularly in our case where fish oil containing long-chain *n*-3 polyunsaturated fatty acids was fed (Clarke 2000).

Palmitate esterification to triglycerides in this study was an order of magnitude less than glycogen and CO_2 production from glucose, and glucose incorporation into liver lipids was 10 times lower than palmitate esterification. Interestingly, palmitate incorporation into liver lipids did not differ among treatments, whereas glucose incorporation, although lower, was stimulated by an increase in dietary carbohydrate level. This suggests that some dietary glucose is used to provide glycerol-glyceride for fatty acid esterification. Esterification in land animals has been shown to vary with feed intake, rate of fat deposition, level of dietary energy consumed and dietary carbohydrate form (Rule 1995; Gilbert *et al.* 2003). Unfortunately, it is not possible in the current study to distinguish glycerol–glyceride from fatty acids produced from labelled glucose, because ^{14}C was measured in total lipid only.

If glycolytic activity in hybrid striped bass liver is minimal in response to 200 and 400 g kg^{-1} dietary carbohydrate, then the high rate of glucose oxidation to $^{14}CO_2$ in comparison to the low rate of incorporation into hepatic lipid or glycogen suggests the majority of glucose in liver is converted to CO_2 via pentose cycle/triose phosphate pathway production of reducing equivalents (NADPH) for lipogenesis. Hilton & Atkinson (1982) first suggested a preference for pentose phosphate pathway utilization in fish when they found increased fructose-1,6-diphosphatase activity, which supports gluconeogenesis or pentose cycle processing, in trout fed high-carbohydrate diets. The fact that glucose incorporation into liver lipid was responsive to dietary treatments in our study strengthens this notion. Although the use of uniformly ^{14}C-labelled glucose does not allow unequivocal distinction of glycolytic versus pentose cycle produced CO_2, which derive from different carbons in glucose, these results are in general agreement with *in vivo* findings in hybrid striped bass. Bequette *et al*. (2006) applied mass isotopomer distribution analysis to the study of dietary starch use (uniformly ^{13}C-labelled dextrin) in hybrid striped bass when included at 20% of diet. In examining carbon flows into and out of the Krebs cycle, flux of glucose carbon to acetyl-CoA was minor compared to other substrates, i.e. glucose was not a major oxidative substrate for this fish. Furthermore, amino acids rather than glucose were the major source of carbon in the anaplerotic reaction which replenishes OAA in hybrid striped bass. However, it is still unclear whether at higher (>200 g kg^{-1}) carbohydrate intake, glucose flux in hybrid striped bass can be shifted towards oxidative or anaplerotic roles, thus sparing amino acids. The results of this study suggest otherwise.

The present data indicate long-term adaptation resulted in an upregulation of glucose catabolism to CO_2 as dietary carbohydrate increased from 200 to 400 g kg^{-1} of diet. However, carbohydrate complexity in the diet did not appear to alter this mechanism. Because fish are unable to rapidly sequester circulating glucose, the proposed mechanism for improved carbohydrate utilization with increased carbohydrate complexity in the diet is that digestion and absorption will be slowed, thus reducing the hyperglycaemic response. This would allow for a more uniform delivery of glucose to the tissues over time and enable them to utilize circulating glucose as a fuel or anabolic substrate. It is notable that CO_2 production was substantially lower, though not statistically significant, in explants from fish fed the more complex carbohydrates. This may be an artefact of the *in vitro* methodology which only captures an instant in time. If the enzymes associated with glucose catabolism were elevated uniformly as carbohydrate level increased, then we would overlook affects on metabolism by carbohydrate complexity. Bequette *et al*. (2006) suggested that oxidation of glucose to pyruvate in hybrid striped bass is not nearly as limited as the enzymatic pathways that control entry of pyruvate into the Krebs cycle. Hence, the range of effect of varying dietary carbohydrate complexity on complete glucose oxidation, i.e. entry into the Krebs cycle as acetyl-CoA, may be extremely limited in this fish.

If glucose is not a major oxidative substrate for hybrid striped bass, then the level of carbohydrate included in the diet is an important consideration for formulating metabolically efficient diets. These data suggest hybrid striped bass do not store significant amounts of glycogen in the muscle (S.D. Rawles, unpublished data), but putatively do store significant amounts in liver. As noted previously, nearly half the liver of hybrid striped bass can accommodate glycogen or lipid storage, but the implications of this ability to overall fish health, or the mobility of these reserves during feed deprivation or low oxygen stress are not known. Total storage capacity in liver is obviously limited relative to total dietary carbohydrate intake. Glucose oxidation for immediate energy appears to be low in these fish, while amino acid sparing for muscle accretion at higher dietary levels of carbohydrate is doubtful. Nevertheless, diet derived glucose may also be used to synthesize fatty acids *de novo* or used to create energy to drive the processes of fatty acid synthesis. However, fat-laden fish are a detriment to continued success in the market. The best growth performance and body condition indices were achieved when hybrid striped bass were fed diets containing 110–220 g kg^{-1} dietary carbohydrate and nearly equal caloric quantities of lipid (Gaylord & Gatlin 2000). This is similar to other findings that a balance between dietary lipid and carbohydrate maximizes protein sparing in carnivorous fish as long as carbohydrate levels in the diet are kept reasonably low (Hemre *et al*. 2002). In view of these observations, it appears that the level of soluble carbohydrate in the diet of hybrid striped bass should be limited to 200 g kg^{-1} or less. Considering typical analyses of feedstuffs commonly included in practical fish diets, no more than about 300–350 g kg^{-1} milled grain products should be included in the diet for hybrid striped bass for maximum metabolic efficiency.

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